PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 10796-053	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/CA2004/002118	International filing date *(day/month/year)* 13 December 2004 (13-12-2004)	Priority date *(day/month/year)* 12 December 2003 (12-12-2003)

International Patent Classification (IPC) or national classification and IPC
IPC: ***C12Q 1/68*** (2006.01)

Applicant
INFECTIO RECHERCHE INC. ET AL

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 7 sheets, as follows:
      [X] sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. 1 and the Supplemental Box.
   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - [X] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [ ] Box No. IV Lack of unity of invention
   - [X] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [ ] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [X] Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12 October 2005 (12-10-2005)	26 April 2006 (26-04-2006)
Name and mailing address of the IPEA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Authorized officer Qianfa Chen (819) 994-1374

Box No. I Basis of the report

1. With regard to the **language,** this report is based on:

[X] the international application in the language in which it was filed

[ ] a translation of the international application into , which is the language of a translation furnished for the purposes of:

[ ] international search (Rules 12.3(a) and 23.1(b))

[ ] publication of the international application (Rule 12.4(a))

[ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

[ ] the international application as originally filed/furnished

[X] the description:

[X]	pages	1-34		as originally filed/furnished
[ ]	pages*		received by this Authority on	
[ ]	pages*		received by this Authority on	

[X] the claims:

[X]	pages	35-37 containing claims 1-22 and 23 (partial)		as originally filed/furnished
[ ]	pages*		as amended (together with any statement) under Article 19	
[X]	pages*	38-44 containing claims 23 (partial) and 24-71	received by this Authority on	12 October 2005 under Article 34
[ ]	pages*		received by this Authority on	

[X] the drawings:

[X]	pages	1/2-2/2		as originally filed/furnished
[ ]	pages*		received by this Authority on	
[ ]	pages*		received by this Authority on	

[X] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/figs

[ ] the sequence listing *(specify):*

[ ] any table(s) related to sequence listing *(specify):*

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/figs

[ ] the sequence listing *(specify):*

[ ] any table(s) related to sequence listing *(specify):*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-71	YES
	Claims		NO
Inventive step (IS)	Claims		YES
	Claims	1-71	NO
Industrial applicability (IA)	Claims	1-71	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Reference is made to the following documents cited in the International Search Report and the document number is consistent with the document number used in the Written Opinion:

D1. US 2003/0152995 A1 (HANNAH, E.), 14 August 2003.
See abstract; and paragraphs 55 and 63.

D4. US 6,197,949 B1 (TEOULE, R. et al.), 6 March 2001.
See abstract; column 2, lines 5-19; column 2, lines 56-61; column 6, lines 28-35; and column 7, lines 8-16.

D5. WO 02/095052 (HYLDIG-NIELSEN, J. et al.), 28 November 2002.
See abstract; page 2, lines 20-25; and page 8, line 14 to page 9, line 2.

D8. WO 02/081735 A3 (LECLERC, M. et al.), 17 October 2002.
See abstract; and page 2, line 24 to page 4, line 16.

D1 describes an apparatus, composition and related method for sequencing a target nucleic acid using peptide nucleic acids (neutral) as probes (paragraph 55), wherein one or more labels may be attached to each probe. A label may be detected by using a variety of means, such as spectrophotometer, luminometer, NMR, mass-spectroscopy, imaging systems, photo multiplier tube, and/or other appropriate standard detection means. In certain embodiments conductive polymers may be used as label. Conductive polymers are tunable to unique spectroscopic profiles based on the polymer composition, length, side chain groups and/or dopants (paragraph 58). Typical conductive polymers include, but not limited to polyaniline, polyphenylene-vinylene, polythiophene etc. (paragraph 63).

D4 describe a method for detecting hybridization of nucleic acids using a copolymer (column 2, lines 5-19) comprising an electrically conductive polymer (e.g., polythiophene, column 2, lines 56-61) and a nucleotide, an oligonucleotide or one of the analogues thereof (e.g., analogues of the sugar-phosphate chain such as mono- or dithiophosphates, methylphosphonates and phosphotriesters, column 6, lines 28-35).

[Continuation in Supplemental Box]

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

D. Description Defects

The description does not comply with Article 5 of the PCT. A statement in an application, such as found on page 6, line 9, and page 21, line 6, which incorporates by reference any other document, does not comply with Article 5 PCT. The description should be complete in and of itself. A person skilled in the art should be able to understand the patent specification without reference to any other document.

The description does not comply with Article 5 of the PCT. Specifically, all documents referred to in the description must be available to the public. Reference to the documents on pages 5, lines 11 and 21, page 9, line 31, and page 27, line 16, must be deleted or replaced by their corresponding publication numbers.

E. Claim Defects

Claims 1, 28, 54, 60 and 64 are broader in scope than the teaching of the description and do not comply with Article 6 of the PCT. The expression "uncomplexed neutral capture probe" encompasses probes that are not contemplated in the description by the applicant. The description only describes the use of peptide nucleic acids or methylphosphonates as the neutral capture probe. Therefore, applicant should define the "neutral capture probes" accordingly.

Claims 1, 28, 54, 60 and 64 do not comply with Article 6 of the PCT. A metal atom, a molecule and a macromolecule cannot be appropriate members of a single group.

Supplemental Box relating to Sequence Listing

Continuation of Box No.1, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

      [X] a sequence listing

      [ ] table(s) related to the sequence listing

   b. format of material

      [X] on paper

      [X] in electronic form

   c. time of filing/furnishing

      [ ] contained in the international application as filed

      [ ] filed together with the international application in electronic form

      [X] furnished subsequently to this Authority for the purposes of search and/or examination

      [ ] received by this Authority as an amendment* on

2 [ ] In addition, in the case that move than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. 1 applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded".*

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of: Box No. V (page 1 of 2)

D5 describes a method for detecting target nucleic acids using peptide nucleic acid as probe (abstract and page 2, lines 20-25), wherein the detectable moieties that can be used to label PNA probes can include an enzyme, such as alkaline phosphatase (page 8, line 14 to page 9, line 2).

D8 describes a method for the simple optical and electrochemical detection of a target nucleic acid using a DNA probe in the presence of a water-soluble, cationic polythiophene derivative (a positively charged reporter), wherein the detection method is based on different electrostatic interactions between the water-soluble, cationic polythiophene derivatives and single-stranded or double-stranded (hybridized) oligonucleotides. D8 describes the use of negatively or positively charged probes. D8 does not describe the use of a neutral capture probe (e.g., a peptide nucleic acid).

A. Novelty

Claims 1-71 meet the criteria set out in Article 33(2) of the PCT, because the closest prior art (D1, D2 or D8) does not teach a method for the detection of the presence of a nucleic acid target using neutral capture probes (i.e., peptide nucleic acids) in the presence of a positively charged reporter (e..g., a water-soluble, cationic polythiophene derivative) wherein the positively charged reporter is added to the hybridization mixture during the hybridization or after the formation of the hybrids between the neutral capture probes and the nucleic acid targets.

B. Inventive Steps

Claims 1-71 do not comply with Article 33(3) of the PCT. The subject matter of these claims would have been obvious on the claim date to a person skilled in the art or science to which it pertains having regard to D1 or D4, combined with D8 (claims 1, 2, 13, 17-19, 23, 24, 27-29, 40, 44, 45, 49, 50, 53-55, 60-62, 64-66 and 68-70), and further combined with D5 (claims 25 and 51) and common general knowledge (claims 3-12, 14-16, 20-22, 26, 30-39, 41-43, 46-48, 52, 56-59, 63, 67 and 71). D1 or D4 separately describes a method or kit for the simple optical and electrochemical detection of a target nucleic acid using neutral capture probes (e.g., peptide nucleic acids or methylphosphonates) labelled with a positively charged reporter (e.g., polythiophene derivative). D1 or D4 does not describe a detection method wherein the positively charged reporter is added to the hybridization mixture during the hybridization or after the formation of the hybrids between the neutral capture probes and the nucleic acid targets. However, D8 describes a method for the simple optical and electrochemical detection of a target nucleic acid using nucleic acid probes, wherein the positively charged reporter is added to the hybridization mixture during the hybridization or after the formation of the hybrids between the neutral capture probes and the nucleic acid targets. Therefore, it would be obvious to a person skilled in the art to substitute the labelled neutral capture probe in hybridization-based detection method of D1 or D4 with an unlabelled neutral capture probe for the detection of nucleic acid targets in the presence of a positively charged reporter that is added to the hybridization mixture during the hybridization or after the formation of the hybrids between the neutral capture probes and

[Continuation in Supplemental Box]

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of: Box No. V (page 2 of 2)

the nucleic acid targets, as taught by D8. Having done so, said skilled person would have a reasonable expectation of success of arriving at the subject matter of claims 1, 2, 13, 17-19, 23, 24, 27-29, 40, 44, 45, 49, 50, 53-55, 60-62, 64-66 and 68-70. Unless a surprising result is demonstrated over the method of D1 or D4, an inventive step cannot be acknowledged for claims 1, 2, 13, 17-19, 23, 24, 27-29, 40, 44, 45, 49, 50, 53-55, 60-62, 64-66 and 68-70. With respect to claims 25 and 51, D5 describes a method for detecting target nucleic acids using a peptide nucleic acid as probe and an enzyme such as an alkaline phosphatase as reporter. Therefore, claims 25 and 51 lack an inventive step. Further, claims 3-12, 14-16, 20-22, 26, 30-39, 41-43, 46-48, 52, 56-59, 63, 67 and 71 refer to technical features that are routinely used in the detection of a nucleic acid. The inclusion of such technical features does not involve any inventive ingenuity.

comprise polythiophenes.

24. A method according to claim 23, wherein said polythiophenes are water soluble and cationic.

25. A method according to claim 1, wherein said reporters comprise enzymes.

26. A method according to claim 25, wherein said enzymes comprise alkaline phosphatase having polystyrene beads conjugated thereto.

27. A method according to claim 1, wherein said detection is selected from the group consisting of optical detection, fluorometric detection, colorimetric detection, electrochemical detection, chemiluminescent detection, microscopy and spectrophotometric detection.

28. A method for detecting the presence of nucleic acids in a sample, said method comprising:

(a) exposing uncomplexed neutral capture probes to a sample possibly containing complementary nucleic acid targets and containing positively charged reporters selected from group consisting of transition metal atoms, molecules and macromolecules, thereby generating a mixture;

(b) submitting said mixture to hybridization conditions which provide for said nucleic acids targets to bind specifically to complementary neutral capture probes, thereby generating negatively charged capture probe-nucleic acid target hybrids, said reporters being capable of electrostatically binding to said hybrids, thereby generating higher-order complexes; and

(c) detecting said higher-order complexes.

29. A method according to claim 28, wherein said nucleic acids targets are unlabeled.

30. A method according to claim 1, wherein said capture probes are immobilized on a support surface.

31. A method according to claim 30, wherein said support surface is selected from the group consisting of a glass surface, a silicon surface, a gold surface, an electrode surface, a particle surface, a gel matrix, a membrane surface, a paper surface and a plastic surface.

32. A method according to claim 30, wherein said support surface comprises a solid support surface

33. A method according to claim 32, wherein said solid support surface comprises a probe array.

34. A method according to claim 30, wherein said neutral capture probes are chemically modified to incorporate a functional group providing for said probes to covalently link to said support surface.

35. A method according to claim 34, wherein said functional group is selected from the group consisting of amine, aldehyde, thiol, epoxy or carboxyl moieties.

36. A method according to claim 30, wherein said support surface is coated with a passivation agent preventing non-specific binding of nucleic acid targets.

37. A method according to claim 36, wherein said passivation agent is selected from the group consisting of polyvinylpyrollidone, polyethylene glycol, and BSA.

38. A method according to claim 30, wherein said support surface is chemically modified, to facilitate coupling and chemical bonding of said neutral probe to said support surface.

39. A method according to claim 38, wherein said support

surface is chemically modified to contain functional groups selected from the group consisting of an aldehyde, an aminoalkylsilane activated with carbonyldiimidazole, thiol, epoxy or carboxyl moieties.

40. A method according to claim 28, wherein said neutral capture probes are selected from the group consisting of peptide nucleic acids (PNA), and methylphosphonate.

41. A method according to claim 28, wherein said nucleic acid targets are selected from the group consisting of DNA and RNA molecules.

42. A method according to claim 28, wherein said nucleic acid targets are generated by methods selected from the group consisting of polymerase chain reaction (PCR), reverse transcriptase-PCR (RT-PCR), strand displacement amplification (SDA), ligase chain reaction (LCR), transcription-associated amplification, nucleic acid sequence-based amplification (NASBA), whole genome amplification (WGA), helicase-dependent isothermal amplification, and chemical synthesis.

43. A method according to claim 28, further comprising a washing step after step (b).

44. A method according to claim 28, wherein said reporters exhibit low affinity for uncharged probes

45. A method according to claim 28, wherein said reporters are capable of electrostatically binding to the phosphate backbone of said hybrids.

46. A method according to claim 28, wherein said transition metal atoms are selected from the group consisting of Ag^{+} and Cd^{++}.

47. A method according to claim 28, wherein said transition metal atoms comprise ions that can be chemically modified to yield higher-order complexes using bound nucleic acids as a scaffold.

48. A method according to claim 28, wherein said detection includes a chemical reaction step rendering said transition metal cations detectable.

49. A method according to claim 28, wherein said reporters comprise polythiophenes.

50. A method according to claim 49, wherein said polythiophenes are water-soluble and cationic.

51. A method according to claim 28, wherein said reporters comprise enzymes.

52. A method according to claim 51, wherein said enzymes comprise alkaline phosphatase having polystyrene beads conjugated thereto.

53. A method according to claim 28, wherein said detection is selected from the group consisting of optical detection, fluorometric detection, colorimetric detection, electrochemical detection, chemiluminescent detection microscopy and spectrophotometric detection.

54. A kit for detecting the presence of nucleic acids in a sample, said kit comprising:

uncomplexed neutral capture probes;

a control sample possibly containing nucleic acid targets that are complementary to the neutral capture probes; and

one or more positively charged reporters selected from the group consisting of transition metal cations, molecules or macromolecules; said reporters being capable of electrostatically binding to negatively charged capture probe-nucleic acid target hybrids.

55. A kit according to claim 54, wherein said neutral capture probes are selected from the group consisting of peptide nucleic acids (PNA) and methylphosphonate.

56. A kit according to claim 54, wherein said capture probes are immobilized on a support surface.

57. A kit according to claim 56, wherein said support surface is selected from the group consisting of a glass surface, a silicon surface, a gold surface, an electrode surface, a particle surface, a gel matrix, a membrane surface, a paper surface or a plastic surface.

58. A kit according to claim 56, wherein said support surface comprises a solid support surface support

59. A kit according to claim 58, wherein said solid support surface comprises a probe array.

60. A method for detecting the presence of nucleic acids in a sample, said method comprising:

(a) exposing uncomplexed and unlabeled neutral capture probes to a sample possibly containing unlabeled complementary nucleic acid targets, thereby generating a mixture;

(b) submitting said mixture to hybridization conditions which provide for said nucleic acids targets to bind specifically to complementary neutral capture probes, thereby generating negatively charged capture probe-nucleic acid target hybrids;

(c) adding said negatively charged hybrids to positively charged reporters selected from group consisting of transition metal atoms, molecules, and macromolecules being capable of electrostatically binding to said hybrids, thereby generating higher-order complexes; and

(d) detecting said higher-order complexes.

61. The method of claim 60, wherein said positively

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charged reporter comprises a polythiophene.

62. The method of claim 61, wherein said polythiophene is a positively-charged water-soluble polythiophene derivative.

63. The method of claim 60, wherein said neutral capture probes are immobilized at the surface of a solid support.

64. A method for detecting the presence of nucleic acids in a sample, said method comprising:

(a) exposing uncomplexed and unlabeled neutral capture probes to a sample possibly containing unlabeled complementary nucleic acid targets and containing positively charged reporters selected from group consisting of transition metal atoms, molecules and macromolecules, thereby generating a mixture;

(b) submitting said mixture to hybridization conditions which provide for said nucleic acids targets to bind specifically to complementary neutral capture probes, thereby generating negatively charged capture probe-nucleic acid target hybrids, said reporters being capable of electrostatically binding to said hybrids, thereby generating higher-order complexes; and

(c) detecting said higher-order complexes

65. The method of claim 64, wherein said positively charged reporter comprises a polythiophene.

66. The method of claim 65, wherein said polythiophene is a positively-charged water-soluble polythiophene derivative.

67. The method of claim 64, wherein said neutral capture probes are immobilized at the surface of a solid support.

68. A kit for detecting the presence of nucleic acids in a sample, said kit comprising:

uncomplexed and unlabeled neutral capture probes;

a control sample possibly containing unlabeled nucleic acid targets that are complementary to the neutral capture probes; and

one or more positively charged reporters selected from the group consisting of transition metal cations, molecules or macromolecules; said reporters being capable of electrostatically binding to negatively charged capture probe-nucleic acid target hybrids.

69. The kit of claim 68, wherein said positively charged reporter comprises a polythiophene.

70. The kit of claim 69, wherein said polythiophene is a positively-charged water-soluble polythiophene derivative.

71. The kit of claim 68, wherein said neutral capture probes are immobilized at the surface of a solid support.